Surprising Twists to Exocyst Function

What do neurons use the exocyst complex for? In this issue of *Neuron*, using mutations in one exocyst component, Mehta et al. reach the surprising conclusion that exocyst function is divisible: different components play distinct roles. These studies also suggest that the exocyst may regulate membrane insertion of cell adhesion molecules required for synaptic partner choice.

The complex cellular architecture unique to neurons poses significant cell biological questions. This specialized architecture reflects three fundamental properties: morphological complexity, intricate patterns of synaptic connectivity, and synaptic vesicle release. Each of these adaptations requires regulated transport of particular proteins to specific subcellular regions, and extensive effort has examined the molecular functions directly involved in each process. However, such studies raise the question of how the underlying trafficking events are themselves controlled. How do neurons put what they need where and when they need it?

Recent work in *Drosophila* analyzing the phenotypic effects of mutations in exocyst complex genes has begun to provide answers. Remarkably, it appears that genes in this complex play sophisticated roles in developmentally regulated membrane trafficking in both neurons and nonneuronal cells. Indeed, emerging evidence suggests that membrane trafficking by exocyst components can play roles in the formation of specific patterns of synaptic connections, the assembly of specialized cell membranes during oogenesis, and the development of apical specializations in photoreceptors. Moreover, it appears that not all members of the complex are equivalent: some appear to completely disrupt complex function, while another subunit may, in fact, have independent functions.

The exocyst, also known as the Sec6/8 complex, was initially identified through genetic experiments in yeast, as well as biochemical experiments in mammalian cells (reviewed in Hsu et al., 1999). In yeast, the exocyst complex genes comprise *sec3*, *sec5*, *sec6*, *sec8*, *sec10*, *sec15*, *exo70*, and *exo84*. Each of these activities is critical for the spatially and temporally regulated fusion of transport vesicles associated with sites of membrane addition during cell growth and division. However, until recently, the roles of this complex in higher eukaryotic cells, and neurons in particular, were almost entirely unknown. What do neurons use the exocyst for?

Knocking out the functions of *sec5* and *sec6* in *Drosophila* was immediately informative (Murthy et al., 2003; Murthy et al., 2005; Beronja et al., 2005). Homozygous mutant animals are born, but arrest and die during larval development once maternal gene products are depleted. More intriguing, though, was the observation that synaptic transmission, at least in *sec5* mutants at specific neuromuscular junctions, was normal, demonstrating that exocyst functions can be separated from those of the traditional SNARE complex. When a novel, in vivo measure of protein trafficking was used, *sec5* and *sec6* mutant larvae displayed significant deficits in the insertion of membrane proteins into the plasma membrane (Murthy et al., 2003; Murthy et al., 2005). These defects were not restricted to developing neurons: germline clones mutant for either *sec5* or *sec6* also displayed extensive defects in membrane insertion (Murthy and Schwarz, 2004; Murthy et al., 2005; Beronja et al., 2005). Finally, photoreceptor cells with reduced levels of *sec6* activity displayed defects in the trafficking of membrane proteins into the rhabdomere, a microvillar specialization on the apical surface (Beronja et al., 2005). Taken together, these studies demonstrate that the exocyst plays broad roles in the insertion of many proteins into the plasma membrane. Since null mutations in *sec5* and *sec6* cause largely indistinguishable phenotypes, these data suggested that the components of the exocyst act in a common pathway essential to cellular viability.

Recent studies on *sec15* suggest a more complex view (Mehta et al., 2005 [this issue of *Neuron*]). Mutations in *sec15* were initially isolated in a genetic screen that began with a high-throughput behavioral assay using somatic mosaic flies in which only photoreceptor cells were rendered homozygous mutant, while the rest of the fly was largely heterozygous. This phototaxis assay identified flies that had diminished vision; by subsequently excluding mutations that affected eye development, and by retaining mutations that affected the electrophysiological response of the retina to flashes of light, it was possible to identify a very wide range of genes whose activities are required for photoreceptor function. With this approach, two alleles of *sec15* were identified, making clear that *sec15* function is fundamentally different from that of *sec5* and *sec6*: photoreceptors homozygous for loss-of-function mutations in *sec15*, but not *sec5* or *sec6*, survive. Indeed, photoreceptors homozygous for *sec15* do many things normally, as they express fate-appropriate transcription factors, differentiate normally, and make apparently normal axon extensions into the brain. However, these cells do display significant defects in their ability to select appropriate synaptic partners.

Photoreceptor synaptic specificity has been studied extensively, with two broad classes of photoreceptors making specific connections in two different brain regions (reviewed in Clandinin and Zipursky, 2002). Axons from six photoreceptors, designated R1–R6, select targets in the first optic ganglion, the lamina. Here, each axon makes synaptic connections with a specific group of target neurons, with the R1–R6 axons from each individual ommatidium selecting targets arranged in precise relative positions. R7 and R8 axons, on the other hand, extend through this brain layer without making any synaptic contacts, and instead choose targets in arranged in two distinct layers within the second optic ganglion, the medulla. In this context, what does *sec15* do?

sec15 mutant photoreceptors displayed defects in both the choice of targets made by R1–R6 cells as well as defects in the layer-specific targeting of R7 and R8. Intriguingly, ultrastructural analysis of R1–R6 terminals revealed synaptic structures that were normal, albeit formed with, presumably, inappropriate targets. These

synapses were functional: *sec15* mutant photoreceptors can elicit light-induced electrical responses from their postsynaptic targets. With these defects in mind, Mehta and coworkers tested the hypothesis that *sec15* mutant cells might fail to choose their synaptic partners because of defects in trafficking of specific cell adhesion and signaling molecules. Indeed, defects in axonal expression were observed for two cell surface molecules, Chaoptin and fasciclin II, as well as for the adherens junction component Armadillo/β-Catenin. Notably, the localization and expression of other molecules, including the cadherins N-Cadherin and Flamingo, both of which are required for photoreceptor target selection, were apparently normal. These experiments make clear that Sec15 function is cargo specific but unfortunately do not link a specific adhesion molecule to the targeting phenotype.

Protein localization studies suggest additional complexity. Sec15 is highly colocalized, though not completely overlapping, with Sec5 in the lamina; a similar expression pattern is also observed with Sec8. Sec6, on the other hand, appears to be largely excluded from photoreceptor axons but is expressed by the postsynaptic target neurons. Consistent with Sec5, Sec8, and Sec15 being part of some larger functional unit, expression of Sec5 and Sec8 is disrupted in photoreceptor axons mutant for *sec15*. As one might expect, expression of Sec6 in lamina neurons is unaffected by the loss of *sec15* function in photoreceptors. Earlier studies of *SEC* gene function in the germline concluded that localization of Sec5 and Sec6 was codependent and that proper localization of Sec8 required Sec5 and Sec6 (Murthy et al., 2005). Taken together, these results are consistent with a role for Sec15 in assembling a subcomplex containing some, but not all, exocyst components in photoreceptor terminals.

How should we think about exocyst function? The exocyst is likely a multipurpose tool, in which different components are recruited to transport particular cargoes (Figure 1). In this view, Sec5 and Sec6 are critical to many functions of the complex, perhaps reflecting the trafficking of many proteins to multiple subdomains. The expression data outlined here, indicating that Sec5 and Sec6 can be expressed in different cells in specific brain regions, do not necessarily mean that these two genes function independently; in many cells, the two genes are coexpressed and have similar phenotypes. Sec15, on the other hand, may only be required to transport specific proteins. One extreme possibility is that this function of Sec15 is independent of other exocyst components; a more reasonable alternative given the expression data is that some, or even all, of the other components are also required. Indeed, it seems likely that Sec5 and Sec6 also act during synaptic target choice, but analysis of this function has so far been thwarted by the requirement for these genes in maintaining cell viability. A related question surrounds the breadth of Sec15 function. Does Sec15 contribute to other exocyst roles? If it does, other components must, at a minimum, be able to compensate for the loss of Sec15 in those functions necessary for cell viability. Clearly further genetic analysis of additional complex members will be necessary.

These studies also add to our understanding of the

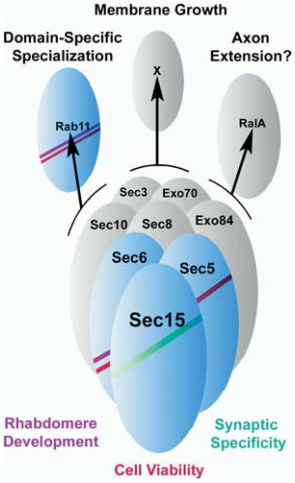

Figure 1. The Exocyst Complex Is Recruited by Different GTPases to Mediate a Variety of Functions

Subunits that have been studied using loss-of-function mutations in *Drosophila* are indicated in blue; phenotypes associated with these manipulations are indicated (hash marks). Components that have been associated with exocyst functions in other contexts are shaded gray.

regulated steps in synaptic partner choice. That is, while much of the axon guidance and targeting field is devoted to characterizing cell surface molecules and their signaling mechanisms, the cell biology of the growth cone is also critical. These studies of the exocyst make clear the importance of regulated trafficking of guidance molecules as a necessary step in specifying the correct pattern of axonal connections, and imply that the regulated activation of exocyst components might be an important step. Exocyst recruitment in response to cadherin-mediated adhesive interactions is an early step in the assembly of junctions between epithelial cells (Yeaman et al., 2004; Grindstaff et al., 1998). Intriguingly, N-cadherin-mediated interactions between photoreceptor axons and their targets are required for photoreceptor axons to innervate the appropriate cartridge (Prakash et al., 2005). Perhaps N-cadherin recruits Sec15 as a critical part of this stabilization process. If so, analyzing the targeting phenotype of *sec15* mutants in greater detail might reveal additional similarities between the phenotypes displayed by *sec15* and *N-cadherin* mutant photoreceptors.

Finally, exocyst components are recruited to the membrane by small GTPases, including those of the Ral and Rab families (Moskalenko et al., 2002; Shipitsin and Feig, 2004; Zhang et al., 2004). Indeed, Rab11 function is critical to rhabdomere development in *Drosophila* photoreceptors, and Rab11 interacts with Sec5 in these cells (Satoh et al., 2005; Beronja et al., 2005). Moreover, RalA has been implicated in filopodial outgrowth in response to cytokine signaling (Sugihara et al., 2002). However, the roles, if any, of these small GTPases during axonal development remain unexplored. Perhaps examining these molecules in greater detail will enable us to define a sequence of events in which exocyst recruitment and activation are critical to

the formation of new axon extensions. An intriguing possibility is that the exocyst might provide another link between guidance receptors, small GTPases, and the downstream changes in growth cone shape that ultimately determine the morphology and connectivity of a neuron.

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